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Gut microbiome in gestational diabetes: a cross-sectional study of mothers and offspring 5 years postpartum

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Key words
Gut microbiome, gut microbiota, gestational diabetes mellitus, pregnancy, children

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Conflict of interest
The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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Abstract

Introduction. An altered gut microbiome composition is shown to be associated with various diseases and health outcomes. We compare the gut microbiota of women who developed gestational diabetes mellitus (GDM) with that of those who did not, and the gut microbiota of their offspring, to determine any differences in the composition and diversity of their gut microbiota, which may be correlated with their GDM state. *Material and methods.* All women were at high risk for GDM and participated in the Finnish Gestational Diabetes Prevention Study (RADIEL). Stool samples were obtained, 5 years postpartum, from 60 GDM-positive women, 68 non-GDM control women, and their children (n = 109), 237 individuals in total. 16S ribosomal RNA gene sequencing was employed to determine the composition of bacterial communities present. Statistical correlations were inferred between clinical variables and microbiota, while taking into account potential confounders. *Results.* In mothers, no significant differences were observed in microbiota composition between the two groups. Genus *Anaerotruncus* was increased in children of women with GDM ($p < 0.001$). Beta-diversity measures showed that a mother and her child have a more similar microbiome composition when compared with unrelated children, other mothers, or the children compared with each other ($p < 0.001$). *Conclusions.* These results suggest that there may be no discernible microbiome basis to GDM susceptibility in high-risk women, whereas microbiome differences between the offspring could be of greater biological significance. The heterogeneous nature of the disease could be obscuring potential differences between women. A longer time-series study, with carefully defined subject subgroups, may be an appropriate course of future investigation into GDM and the microbiome.

Abbreviations: 16S, 16S ribosomal RNA gene; GDM, gestational diabetes mellitus; OTU, Operational Taxonomic Unit; PCR, polymerase chain reaction.

Introduction

The incidence of gestational diabetes mellitus (GDM) is on the rise globally (1). GDM can have a long-lasting effect on both the women and their offspring, including an increased risk of developing obesity, metabolic

Key Message

Here we look at the relation between gestational diabetes and the gut microbiome in both mothers and children and suggest potential future avenues of study for this new direction in exploring diabetes mellitus.

syndrome and type 2 diabetes, which may be transmitted via the microbiome (2–5).

Because of its large impact on human health and disease, the microbiome has come under increasing scrutiny over the last decade. The gastrointestinal tract is the best characterized microbiome within the human body and our knowledge of it is constantly expanding. The prevalence of obesity is increasing worldwide, and obesity is a key risk factor for a number of noncommunicable diseases including type 2 diabetes, coronary heart disease and several cancers (6–8). Multiple studies have shown a link between the gut microbiome and obesity, in particular the ratio of the abundances of the phyla Bacteroidetes and Firmicutes (9,10).

Studies investigating the relation between diabetes and the gut microbiome have shown a decreased microbial diversity in people with type 1 diabetes compared with controls, as well as a marked difference in the ratio of Bacteroidetes and Firmicutes abundance between the two groups (11,12). A number of functional differences in the gut microbiota have been observed between people with type 2 diabetes and control subjects, from deficiency in butyrate biosynthesis and glucose metabolism to higher levels of pathogens such as *Escherichia coli*, although some of the differences associated with type 2 diabetes may be driven by medications such as metformin (13–15).

The gut microbiome's influence and importance in the pathogenesis of GDM is yet to be explicitly studied. It has been shown that the microbiome may be severely remodeled during pregnancy with reduced diversity and bacterial richness observed in the third trimester, compared with the first trimester (16). However, this was not confirmed in a more recent study with serial collection of stool throughout pregnancy (17).

Current research linking the gut microbiome to GDM has so far produced very few insights, and the role of the gut microbiome in GDM has yet to be elucidated. Moreover, no study has looked at the long-term effects of GDM on the microbiota of children from GDM pregnancies and whether this might contribute to their increased risk of developing diabetes and other metabolic disorders in the future.

Here we aim to compare the gut microbiota, 5 years postpartum, of women who had been diagnosed with GDM, to that of high-risk women with normal glycemic control originating from the Finnish Gestational Diabetes Prevention Study (RADIEL) (18).

Material and methods

Study subjects and sample collection

This study was based on samples obtained 5 years after delivery from participants in the RADIEL study, a randomized controlled intervention trial between February 2008 and January 2014. The study investigated the effects of a moderate lifestyle intervention on GDM incidence in women who were at high risk of GDM (18). Participants were pregnant women, at or below 20 weeks of gestation, and recruited due to a previous history of GDM and/or a prepregnancy body mass index of ≥ 30 kg/m^2. Exclusion criteria included: diabetes diagnosis before pregnancy, medications affecting glucose metabolism, multiple pregnancies, physical disabilities, current substance use and severe psychiatric disorders (18). All participants consented to the 5-year follow-up study.

For the purposes of this study, stool samples were obtained from RADIEL participants: 60 women diagnosed with GDM during the RADIEL study, 68 non-GDM controls, as well as their children ($n = 109$), in total $n = 237$. As this was a pilot study, we analyzed samples from a similar number of women with GDM during the RADIEL pregnancy and compared them with women not diagnosed with GDM during the RADIEL pregnancy. We included the first 60 women and the first 68 controls from the 5-year follow up for the present study. There were fewer children than mothers included in the analyses due to some children's samples either not being present or there being inadequate amounts of sample material for sequencing.

Participants attended a clinical visit 5 years postpartum. Measurements for glucose tolerance, blood pressure, height, weight, body composition (InBody 3.0: InBody, Seoul, South Korea) were assessed, for both mother and child.

Stool samples were collected at home before the clinical visit, using collection kits preloaded with stool DNA stabilizer (PSP® Spin Stool DNA Plus Kit: Stratec Molecular GmbH, Berlin, Germany) (19). The tubes were then kept in the subject's home freezer until the clinical visit after which they were stored at −80°C.

DNA extraction, polymerase chain reaction and sequencing

PSP® Spin Stool DNA Plus Kits (Stratec Molecular) were used for total DNA extraction. Polymerase chain reaction (PCR) amplification was performed using an ARKTIK Thermal Cycler (Finnzymes Diagnostics- Thermo Fisher Scientific, Finland). Further details in the Supplementary material (Appendix S1).

Final PCR fragments were pooled in equal concentrations and run on a MiSeq Sequencer (Illumina, San Diego, CA, USA) using a v2 600 cycle kit paired-end (325 bp + 285 bp). The complete data set consisted of 28 956 675 raw reads with an average of 121 667 raw reads per sample (mothers: total 15 568 844, average 121 632; children: total 13 387 831, average 121 708).

The DNA extraction, PCR and sequencing steps were carried out at the DNA Sequencing and Genomics Laboratory, University of Helsinki.

Sequence quality control, operational taxonomic unit clustering and taxonomy assignment

CUTADAPT (20) and MOTHUR (21) were used for sequence quality control, Operational Taxonomic Unit (OTU) clustering and taxonomy assignment. Full details are given in the Supplementary material (Appendix S1).

Diversity measurements and statistical data analysis

OTU and taxonomy tables were imported into R (22) for further analysis. The PHYLOSEQ package (23) was used for handling sample metadata, taxonomy and sequence counts.

Alpha diversity was compared using the inverse Simpson and Shannon indices, calculated using PHYLOSEQ. The adonis function from the VEGAN R package (24) was used to test for microbial community compositional differences between groups of samples (beta diversity), using Bray–Curtis dissimilarity. Alpha diversity comparisons were performed on nonrarefied data, beta-diversity comparisons with data subsampled to the number of reads in the sample with the least reads (1359 for mothers, 1962 for children and 1359 for mothers and children together). Kruskal–Wallis and pairwise Wilcoxon rank sum tests were used to test for statistically significant differences between groups. For comparisons of beta diversity between sample pairs, there were 99 related mother–child pairs, and to have equally sized groups, 99 samples were randomly drawn from each of the other groups (mother and unrelated child, pairs of mothers, pairs of children); this sampling was performed 100 times, and the average p-values of these 100 comparisons were considered.

The DESEQ2 package (25) was used to identify taxa that may show abundance differences between cases and controls. To avoid including taxa that may be present in only very few samples and that therefore may produce trivially large effect sizes, OTUs that did not have at least two sequences in at least 10 samples were excluded from these comparisons. The Benjamini–Hochberg multiple comparison correction was applied and adjusted p-values were deemed significant when $p \leq 0.05$. The confounding variables included in the statistical models consisted of age, parity (primiparous or multiparous) and sequencing run (run 1 or run 2) for the mothers and birthweight, sex and sequencing run for the children.

Ethical approval

The present study was approved by the ethics committees of Helsinki University Hospital (29 August 2012, Dnro 78/13/03/03/2011).

Results

Clinical characteristics

The clinical characteristics of the women were similar in both groups, with the exception of plasma glucose levels and body fat percentages (Table 1). Clinical characteristics of the children showed no differences between the groups.

Diversity

No difference in alpha diversity was observed between the GMD and non-GDM women; this was also the case when comparing their offspring (all p-values > 0.5).

To test for community compositional differences between the groups, beta diversity measures were performed, with no significant beta diversity differences being observed between the women ($p = 0.7$) or children ($p = 0.6$).

A pairwise comparison of Bray–Curtis dissimilarity between women and their own children, women and unrelated children, all women, and all children, showed that women and their own children have a microbial community, which is much more similar (Figure 1). The women and their own child group is significantly different from all others ($p < 0.001$; median Bray–Curtis dissimilarity 0.622 for women and their own children, 0.751 for women and unrelated children, 0.729 for mothers compared with other mothers and 0.751 for children compared with other children).

Overall microbiome composition

Looking at the bacterial genera present in the women, over 50% of the microbial community was made up by the four most abundant taxa: *Bacteroides* (28.5%) (mean relative abundance), *Faecalibacterium* (11.1%), *Subdoligranulum* (8.2%) and *Lachnospiracea incertae sedis* (6.9%). 64 taxa were not among the 15 most common genera and had a combined relative abundance of 13.6%. In total, 79 genera were identified. The microbiome profiles for GDM and non-GDM groups were very similar, with only minor differences observed between the two (Figure 2).

The top two genera in children were similar in abundance to their mothers, *Bacteroides* at 32.5% and *Faecalibacterium* at 10.9%. In contrast, the third most abundant genus in mothers, *Subdoligranulum*, is only observed as

Table 1. Clinical characteristics of GDM and non-GDM control groups.

	GDM	non-GDM	*p*-value
Mothers	(*n* = 60)	(*n* = 68)	
Months post delivery	59 (2.4)	59 (2.6)	0.99
Age (years)	39.2 (4.4)	37.7 (5.3)	0.14
Weight (kg)	84.4 (5.3)	92.5 (18.7)	0.07
Height (cm)	166.0 (0.02)	168.0 (0.1)	0.16
Body mass index (kg/m^2)	30.6 (1.8)	32.9 (6.3)	0.16
Waist (cm)	103.3 (4.3)	106.8 (16.5)	0.64
Hip (cm)	111.0 (3.8)	115.5 (12.6)	0.12
Waist/hip ratio	0.9 (0.02)	0.9 (0.06)	0.25
Fat-free mass (kg)	51.3 (1.9)	53.9 (6.8)	0.10
Percent body fat (%)	36.6 (2.5)	40.0 (8.9)	<0.01
Systolic blood pressure (mmHg)	125.2 (3.8)	124.6 (12.0)	0.62
Diastolic blood pressure (mmHg)	79.6 (2.6)	79.0 (10.0)	0.45
Fasting P-glucose (mmol/L)	5.7 (0.5)	4.9 (0.4)	<0.01
2-h glucose (mmol/L)	6.6 (0.7)	5.6 (1.5)	<0.01
Insulin (mU/l)	10.0 (1.9)	9.8 (5.2)	0.63
Fasting HDL (mmol/L)	1.5 (0.1)	1.5 (0.4)	0.87
Fasting triglyceride (mmol/L)	0.9 (0.1)	0.9 (0.4)	0.25
Children	(*n* = 57)	(*n* = 52)	
Age (months) at study	59.2 (3.1)	59.1 (2.8)	0.53
Birthweight (g)	3692 (466)	3634 (521)	0.55
Birth length (cm)	50.6 (2.2)	50.6 (2.4)	0.73
Weight (kg)	20.6 (3.3)	20.1 (2.8)	0.55
Height (cm)	111.5 (5.0)	112.3 (5.1)	0.38
Body mass index (kg/m^2)	16.5 (1.6)	15.9 (1.4)	0.06
Waist (cm)	55.0 (4.4)	54.3 (4.1)	0.44
Head circumference (cm)	51.8 (1.5)	51.7 (1.4)	0.70
Systolic blood pressure (mmHg)	100.6 (7.1)	102.3 (9.3)	0.72
Diastolic blood pressure (mmHg)	62.2 (6.7)	63.2 (7.8)	0.71
Fasting P-glucose (mmol/L)	5.2 (0.8)	5.0 (0.5)	0.31
Insulin (mU/l)	6.3 (5.5)	5.9 (6.2)	0.33
Fasting HDL (mmol/L)	1.6 (0.4)	1.5 (0.3)	0.82
Fasting triglyceride (mmol/L)	0.8 (0.3)	0.9 (0.6)	0.99

GDM, gestational diabetes mellitus; HDL, high-density lipoprotein.
Values are presented as mean (SD). Normality of data was determined using the Shapiro–Wilk test. *p*-values were then obtained by way of *t*-tests or Mann–Whitney *U*-tests as required.

the sixth most abundant taxon in the children at 4%. The relative abundance of the top 15 genera and the overall composition of the GDM and non-GDM groups in children remained very similar, the largest difference being observed in *Prevotella* where the abundance in GDM (8.5%) was almost double that in non-GDM (4.8%) (Figure 2).

Differential abundance analysis

The DESEQ2 package was used to look for specific taxa that may be differentially abundant between the women and the children in each group of subjects.

OTU level. The comparisons did not reveal any OTUs that differed significantly between the GDM and non-GDM groups for either the mothers or the children.

Genus level. No significantly different taxa were identified between GDM and non-GDM mothers when comparing the abundances of genera.

Two genera, *Anaerotruncus* and *Victivallis*, were identified as significantly different between the two groups in children (Figure 3a,b, Table 2). Both of these genera were more abundant in the children of mothers with GDM.

Family level. Comparing the bacterial families present, no significantly different abundances in taxa were identified between the two groups of women. *Victivallaceae* was the only family identified as significantly different in children between the two groups; its distribution was similar to that of the genus *Victivallis* (Figure 3c, Table 2).

Comparisons of further clinical characteristics and GDM subgroups. To identify any differences that may exist in the microbiota of women based upon other clinical characteristics, further DESEQ2 comparisons were run on several variables available in the sample metadata and a number of statistically significantly different taxa were discovered (summarized in the Supplementary material, Table S1 in Appendix S2). A full list of the taxonomy of these specific taxa are included in Supplementary material (Table S2 in Appendix S2).

Comparisons were also run on the individual confounding variables as well as on weight, body mass index, height and cholesterol levels, to see whether any of these variables may be associated with differences in microbiota composition. No significant differences were observed other than those reported in the Supplementary material (Table S2).

Discussion

This study set out to compare the gut microbiota of women who had been diagnosed with GDM, with that of women who had undergone a normoglycemic pregnancy, in addition to comparing microbiota of the offspring arising from the pregnancy 5 years postpartum. Although no

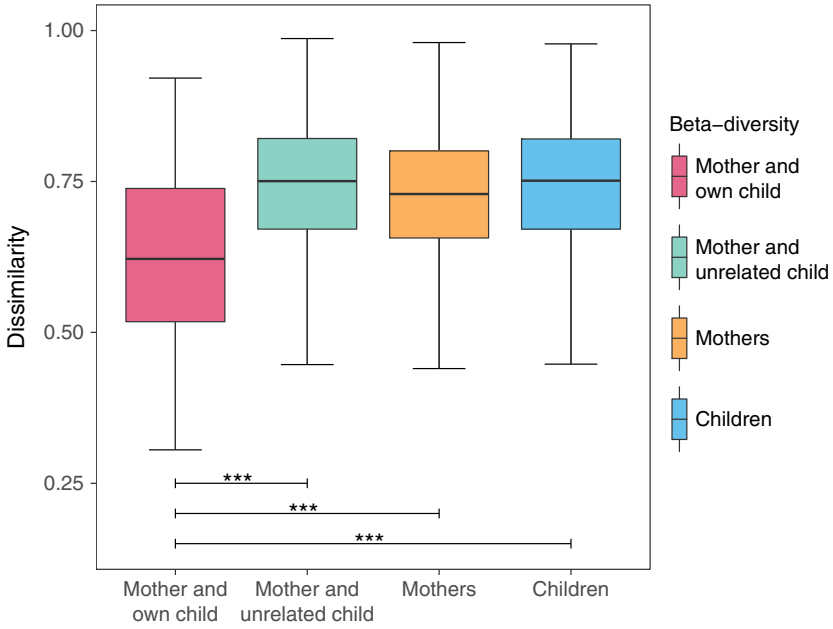

Figure 1. Pairwise Bray–Curtis beta-diversity dissimilarity comparison between mothers and their own children, mothers and unrelated children, all mothers, and all children. Pairwise Wilcoxon test significant *p*-values (based on averages of 100 samplings of 99 pairs from groups other than mother and own child) are overlaid. Mothers and their own children have a microbial community that is much more similar compared with all other comparisons carried out. [Color figure can be viewed at wileyonlinelibrary.com].

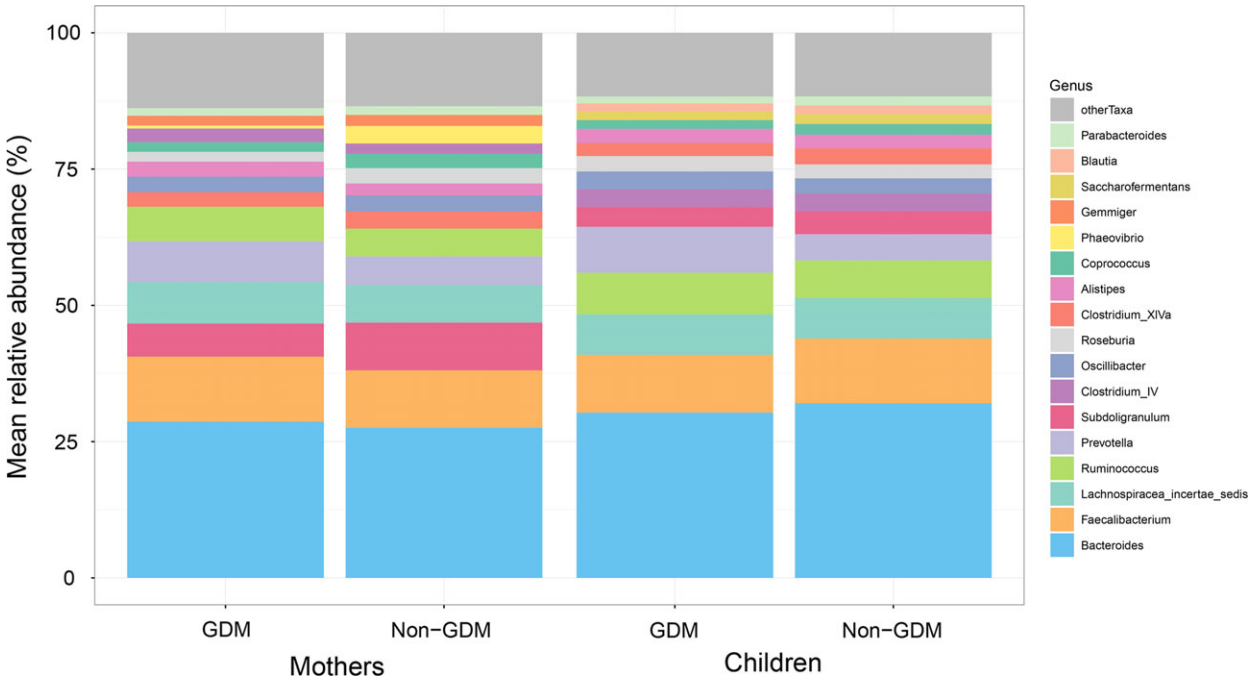

Figure 2. Mean relative abundance of the top 15 genera in gestational diabetes mellitus (GDM) and non-GDM groups. A total of 79 different taxa were observed in mothers with 64 falling under the 'other Taxa' category. A total of 69 different taxa were observed in children with 59 falling under the 'other Taxa' category. [Color figure can be viewed at wileyonlinelibrary.com].

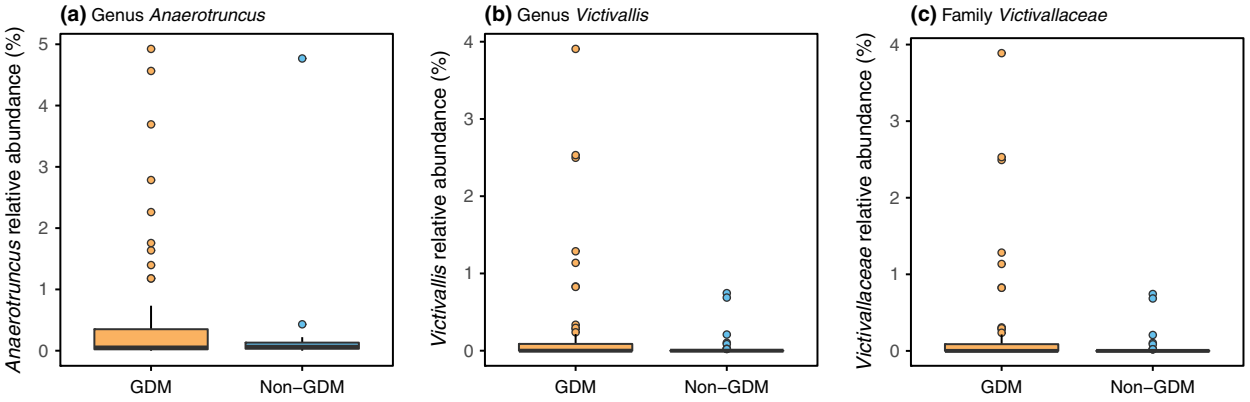

Figure 3. Relative abundances of the three different taxa identified as differentially abundant between the gestational diabetes mellitus (GDM) and non-GDM groups in children. (a) Relative abundance of genus *Anaerotruncus*. (b) Relative abundance of genus *Victiviallis*. (c) Relative abundance of family *Victivallaceae*. [Color figure can be viewed at wileyonlinelibrary.com].

Table 2. Taxa with a statistically significant difference in abundance between the children of GDM and non-GDM mothers.

		GDM			Non-GDM		
Taxon	*p*-value (DESEQ2, fdr-corrected)	Median	First quartile to third quartile	Samples with relative abundance > 0 (%)	Median	First quartile to third quartile	Samples with relative abundance > 0 (%)
Genus *Anaerotruncus*	0.000	0.053	0.023–0.351	76.79	0.065	0.031–0.132	86.54
Genus *Victivallis*	0.005	0.000	0–0.088	33.93	0.000	0–0	15.38
Family *Victivallaceae*	0.004	0.000	0–0.088	33.93	0.000	0–0	15.38

GDM, gestational diabetes mellitus.
Significance defined as false discovery rate adjusted $p < 0.05$ in DESEQ2 comparisons. Medians and interquartile ranges are given for relative abundances (%).

community-wide differences were observed between the post-GDM and non-GDM groups, three specific taxa were identified as differentially abundant in the children.

Baseline characteristics between the groups were similar, except for glucose levels and body fat percentage in the women. The difference in glucose levels between the groups is not unexpected, as women who experience GDM are at higher risk of type 2 diabetes in the future (26).

A pairwise beta-diversity dissimilarity comparison between women and their own children against all women, all children, and women and nonrelated children reveals a significantly greater similarity in the microbial communities in mothers and their own children, compared with the rest. This result is expected, as children are more likely to have a similar microbial community to their mothers, first due to the maternal transfer of microbiota during birth and subsequently via breastfeeding, but second due to the sharing of the same environment as well as nutritional and dietary habits as the child ages, which are key contributors to gut microbiome colonization patterns (27). A child's gut microbiome is thought to undergo significant shifts until approximately 2–3 years of age, after which it is susceptible to fewer changes and represents a more adult-like microbiome (28).

No major differences in the top 15 genera abundances can be seen between the GDM and non-GDM groups in either the women or children. The most abundant taxa are the same in all four groups: *Bacteroides* (phylum Bacteroidetes) and *Faecalibacterium* (phylum Firmicutes). *Bacteroides* has been known as far back as the 1980s to be one of the most abundant genera in the gut, making up approximately 25% of the microbial community (29). *Faecalibacterium* are thought to make up anywhere between 5 and 20% of the gut bacteria in healthy individuals (30). Although Tap et al. report *Faecalibacterium* to be significant butyrate producers, thought to increase insulin sensitivity (31), an increase in *Faecalibacterium* species has been associated with obesity and diabetes (32).

Fugmann et al. (33) have conducted the only study so far aiming to investigate the gut microbiome in women with a recent history of GDM (3–16 months postpartum) including 42 post-GDM and 35 control subjects. Case and control groups, however, differed significantly for

several variables, which may be confounders for GDM, such as adiposity, blood pressure and cholesterol concentrations, and whether these confounders were taken into account in the analysis was not mentioned. In our study, the subjects were much more uniform and similar, and the sample size was also greater.

Results from the study by Fugmann et al. (33) suggest that the Firmicutes phylum is reduced in subjects with GDM compared with controls. In addition, a subset of participants had a microbiome dominated by the *Prevotellaceae* family compared with *Bacteroidaceae* in others. These women were overrepresented in the GDM group, suggesting a shift in the *Bacteroidaceae*/*Prevotella* ratio in GDM. As with most human microbiome research to date, causality cannot be inferred from forming correlations; establishing whether the gut microbiome differences observed in this study are in fact an outcome of disease state is of importance.

Statistical comparisons with DESEQ2 identified two genera and one family as differentially abundant in the children: genus *Anaerotruncus*, genus *Victivallis* and the family *Victivallaceae*.

The *Anaerotruncus* genus was more abundant in the children of women with a history of GDM compared with those whose mothers did not have GDM. This genus has been positively associated with both glucose intolerance and gut permeability, suggesting a role in the pathogenesis of diabetes (34). One study investigating the effects of administering the probiotic yeast *Saccharomyces boulardii* to diabetic mice showed a marked decrease in *Anaerotruncus* abundance in *S. boulardii*-treated mice (35). The probiotic-treated mice displayed lower body weight and fat mass. Hyperglycemia in pregnancy confers a significant increase in risk in the offspring of developing diabetes or obesity in the future (2–4). This could be explained in part by the gut microbiome differences observed in the offspring of women with a history of GDM compared with offspring of women with non-GDM as evidenced here, although further investigation is required to elucidate the full impact of such a difference.

The other differentially abundant taxa observed in children, the genus *Victivallis* and the corresponding family, *Victivallaceae*, were also more abundant in the GDM group. These bacteria were only very recently described (36) and established to separate the *Victivallis* genus from the *Lentisphaera*. *Victivallis* is the sole genus in the family *Victivallaceae*, and although described in 2003 (37), it has not been reported in relation to the gut microbiome and disease, or in fact even fully characterized. It must be noted that the number of samples that represent a difference in abundance between the GDM and non-GDM groups for these bacteria is limited: they are only present in 34% of children in the GDM group and 15% in the non-GDM group, bringing the significance of this result into question.

The results obtained from additional clinical characteristics and microbiota comparisons suggest that further differences in the gut microbiota between the groups may be seen, when using stricter criteria to define the GDM subgroups. The heterogeneous nature of GDM could have resulted in difficulty in observing putative associations between the various features of GDM and the gut microbiome in women.

The present study was based on a convenience sample of 128 women and their children. As this was a pilot study, we aimed at similar numbers of women with GDM and without GDM during the RADIEL-pregnancy 5 years earlier. The women belonging to the non-GDM group in this study have risk factors for GDM. As a result, they are not, perhaps in a traditional way, healthy controls, but are high-risk persons. Therefore, further and larger studies are needed to confirm these findings.

Conclusion

This exploratory study aimed to characterize the gut microbiome differences between women with a history of GDM and nondiabetic, but high-risk, control women, as well as their children, 5 years postpartum. Our findings suggest no differences in the women, although differences between offspring to women with GDM and without GDM could be of greater biological and clinical significance. The most interesting finding is that of the *Anaerotruncus* genus in offspring, which warrants further studies to determine its role in GDM and to investigate whether children with high *Anaerotruncus* levels are at greater risk of developing diabetes or becoming obese when they mature.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Supplemental Methods – additional material and methods information detailing bioinformatics processes.

Appendix S2. Supplemental Results – findings related to the 'additional characteristics' comparisons; Supplemental Tables S1 and S2.